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Natural history and immature stage morphology of *Spialia* Swinhoe, 1912 in the Iberian Peninsula (Lepidoptera, Hesperiidae)

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Abstract. We present new data on the ecology, natural history and geographic distribution of the recently described skipper *Spialia rosae* Hernández-Roldán, Dapporto, Dincă, Vicente & Vila, 2016 and compare its immature stage morphology with the sympatric species *S. sertorius* (Hoffmannsegg, 1804). *Spialia rosae* uses species of *Rosa* L. (Rosaceae) as larval host-plants and prefers montane habitats, while *S. sertorius* feeds on *Sanguisorba minor* Scop. (Rosaceae) and inhabits lower altitudes. *Rosa corymbifera* Borkh. and *R. tomentosa* Sm. are documented for the first time as foodplants of *S. rosae*. We report *Microgaster australis* Thomson, 1895 (Hymenoptera, Braconidae, Microgastrinae) as a larval parasitoid of *S. rosae*. Details of the immature stages of *S. rosae* and *S. sertorius* are shown using scanning electron microscope photographs, confirming the similar immature stage morphology, at least as regards the Iberian *S. sertorius*. In both species, the egg has high radial ribs, the last instar larva has branched setae covering the head, and the pupa has setae with pointed tips, barrel-like cuticular formations, and hairy mesothoracic tubercles. By extensive sampling of the species of *Spialia* in the region of Segovia, Central Spain, we extend the previously known geographic distribution of *S. rosae* to 56 new 100 km^2 MGRS squares, which represents a 155 % increase. *Spialia rosae* is present in the northern part of the interior plateau and in the main mountain systems of the Iberian Peninsula. The main threats to the populations of *S. rosae* are its limited distribution range and the possible effects of climate change due to its specialization in montane habitats. The conservation status of *S. rosae* was previously regarded as Data Deficient (DD). With the addition of new data the species can now be evaluated as Least Concern (LC).

Introduction

The genus *Spialia* Swinhoe, 1912 (Lepidoptera, Hesperiidae) occurs in the Palaearctic region and Africa, and its larvae are generally monophagous or oligophagous on plants of the family Rosaceae (LSPN 1999; Tolman and Lewington 2008; Tshikolovets 2011; Kudrna et al. 2015). The taxonomic

status of the species in this genus has been discussed by different authors (de Jong 1974, 1978; Tolman and Lewington 2008; Tshikolovets 2011; Balletto et al. 2014). According to the latest revision (Hernández-Roldán et al. 2016), the *sertorius* species complex includes at least five species that diverged during the last three million years: *S. orbifer* (Hübner, 1823) in eastern Europe and temperate Asia; the parapatric *S. sertorius* (Hoffmannsegg, 1804) in western Europe and north-western Africa; the allopatric *S. ali* (Oberthür, 1881) from North Africa; *S. therapne* (Rambur, 1832) from Corsica and Sardinia; and the recently discovered cryptic species *S. rosae* Hernández-Roldán, Dapporto, Dincă, Vicente & Vila, 2016 from the Iberian Peninsula, that is sympatric and morphologically similar (in adult wing shape and pattern, and male genitalia, but the female genitalia were not studied) with *S. sertorius*.

Spialia rosae is currently known only from Spain at an elevation range of 860–2640 m, showing a significantly higher altitudinal range than the closely related *S. sertorius* and *S. orbifer*. The species occurs in the main mountain systems of the Iberian Peninsula: Sierra Nevada and La Sagra (Betic Mountains), Iberian Mountain System, Cantabrian Mountains, Central Mountain System and the Pyrenees (Hernández-Roldán et al. 2016).

Spialia rosae can be distinguished from the most closely related species (*S. sertorius*, *S. orbifer*, *S. ali* and *S. therapne*) on the basis of the DNA sequence of the mitochondrial gene cytochrome c oxidase subunit I (COI), the composition of the wing cuticular hydrocarbons and the ecology, suggesting that *S. rosae* represents a case of ecological speciation involving larval host-plant and altitudinal shifts, also apparently associated with a *Wolbachia* infection (Hernández-Roldán et al. 2016). *Spialia rosae* specializes in *Rosa* L. spp. (Rosaceae) as larval host-plants, while the rest of taxa in the group feed on *Sanguisorba* L. spp. (Rosaceae). Larval host-plant choice and mitochondrial markers are independent traits and their 100% match at individual level in sympatry demonstrates the existence of two species based on the Biological Species Concept (Hernández-Roldán et al. 2016). Infection by *Wolbachia* was another character that correlated perfectly with COI and host-plant in sympatry: no infection was detected in any specimen of *S. sertorius*, while all the *S. rosae* specimens tested were infected (Hernández-Roldán et al. 2016).

There are no previous studies on the morphology of the immature stages of the genus *Spialia* based on scanning electron micrographs but, within the Hesperiidae family, there has been a study of the eggs of the subfamily *Pyrginae* (Thust 1997). Morevoer, the morphology of eggs, larvae and pupae of the European species of the genus *Pyrgus* Hübner, [1819] have been extensively studied (Hernández-Roldán et al. 2011, 2012a, 2012b).

At the European level, the conservation status of *Spialia sertorius*, *S. therapne* and *S. orbifer* has been considered as Least Concern (LC) following IUCN criteria (Van Swaay et al. 2010), and *S. rosae* had been included in the category of "Data Deficient" because there is inadequate information to make a direct or indirect assessment of its risk of extinction based on its population status (Hernández-Roldán et al. 2016).

The object of the present paper is to describe the biology, distribution and morphological details of the egg, last instar larva and pupa of *S. rosae* in the Iberian Peninsula and to compare these stages with *S. sertorius*, that partly overlaps in geographic range, in order to test whether these two species show similar immature stage morphology. The morphological study is based on micrographs taken with scanning electron microscopy. We also aim to evaluate the threats and conservation status of the new species in the study area.

Methods

Sample collecting

Spialia eggs were collected in the field by following females until oviposition took place. We also searched for eggs on plants of *Sanguisorba* spp. and *Rosa* spp. Larvae were collected by searching for larval refugia on the foodplants. We collected samples of rose bushes from several localities in order to determine the species. Also, we have been able to study samples of roses from two localities where *S. rosae* was cited in the literature (Monasterio et al. 2017a, 2017b). Species of roses were determined following Calvo and Ross-Nadié (2016). A thorough sampling of the *Spialia* species was made by one of the authors (Vicente, unpublished results) in the province of Segovia (Central Spain) by searching for the species in every 10 × 10 km MGRS square (Military Grid Reference System). The material of both *Spialia* species was only collected in Spain. Coordinates for all the locations were taken using the MGRS system and then converted to geographical coordinates (Degrees and Decimal Minutes, DDM) taken from the centre of the UTM (Universal Transverse Mercator) square.

Rearing took place in the laboratory, using field collected eggs and larvae, and with climatic parameters different from those in the natural habitat. Larvae were provided with the same genus of plants on which they were collected. After emergence of the adults the exuviae were preserved for morphological studies.

Scanning electron micrographs

Images used to study the morphology of the egg, last instar larva and pupa were obtained using a Hitachi S-3000N scanning electron microscope, with an acceleration voltage of 20 kV. Dried samples were used in the case of the egg and pupa. The eggs were killed with ethyl acetate when the larva had already formed inside the egg to prevent the egg collapsing when dried. For the study of pupae, exuviae were used after the emergence of adults. Larvae were preserved in 70% ethanol and then fixed with 1.5% formaldehyde. For larval dehydration a series of increasing concentrations of ethanol was used, ending with absolute ethanol and then the liquid was removed to produce dried samples with an Emitech K850 Critical Point Dryer. Samples were then coated with a 10 nm layer of gold using an Au-Cr Quorum 150TS Sputter. The microscope and all the other equipment used are located in the Servicio Interdepartamental de Investigación (SIDI) of the Universidad Autónoma of Madrid. A scale bar was added to all images. Egg measurements were performed directly on the images using IMAGEJ.1.43 software (Ferreira and Rasband 2010).

Distribution of *Spialia rosae*

The new distributional data for *S. rosae* are based exclusively on immature stages collected on *Rosa* spp., in order to avoid confusion with *S. sertorius*. Both species are morphologically similar as adults, but their larvae feed on different plant genera. Some of the samples in this study were previously used by Hernández-Roldán et al. (2016) and were identified based on the COI mitochondrial DNA marker. The map representing the geographic distribution of *S. rosae*, in 10 × 10 km squares (MGRS system), was drawn with the automatic cartography software QGIS 2.6.1. for WINDOWS (QGIS Development Team 2014). Distributional data were obtained from Hernández-Roldán et al. (2016), Blázquez-Caselles et al. (2016), and Monasterio et al. (2017a, 2017b).

Terminology for the description of wing characters follows García-Barros et al. (2013).

Results

Studied material

Spialia rosae Hernández-Roldán, Dapporto, Dincă, Vicente & Vila, 2016

Spain: 2 Paratype eggs on *Rosa sicula*, Puerto de La Ragua, Sierra Nevada (Granada), 37°5.48'N, 3°3.38'W, 2090 m, 19.vii.2012, leg. et coll. J.L. H.-R. (JHSEM-C19P09); Paratype larva L5 on *Rosa pouzinii*, El Vallecillo, Sierra de Albarracín (Teruel), 40°14.25'N, 1°38.88'W, 1525 m, 5.viii.2012, leg. J.L. H.-R., J.C. Vicente, R. Vila and S. Viader, coll. J.L. H.-R. (JHSEM-C20P01 and JHSEM-C20P02), tissues in coll. IBE (RV--12L066); Male paratype, Puerto de El Cubillo, Tragacete (Cuenca), *ex larva* L3 on *Rosa sicula* 40°19.74'N, 1°45.84'W, 1630 m, 6.viii.2012, leg. J.L. H.-R., J.C.V., R.V. and S.V., coll. J.L. H.-R. (pupal exuviae: JHSEM-C19P12, adult: JH6620), tissues in coll. IBE (RV-12L068); Egg on *Rosa* sp., Aldeavieja (Ávila), 40°46.59'N, 4°28.88'W, 1235 m, 26.viii.2012, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Torreiglesias (Segovia), 41°8.51'N, 4°0.77'W, 915 m, 10.viii.2013, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Pajares de Pedraza (Segovia), 41°8.57'N, 3°53.62'W, 1015 m, 26.x.2013, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Duratón, alrededor del municipio (Segovia), 41°19.48'N, 3°39.43'W, 960 m, 27.x.2013, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Sebúlcor (Segovia), 41°13.97'N, 3°53.70'W, 940 m, 27.x.2013, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Sepúlveda (Segovia), 41°19.43'N, 3°46.60'W, 930 m, 27.x.2013, J. C. V. and B. P. leg.; Egg on *Rosa* sp., El Espinar, Estación (Segovia), 40°46.83'N, 4°7.55'W, 1280 m, 08.vi.2014, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Escobar de Polendos (Segovia), 41°3.04'N, 4°7.83'W, 935 m, 21.vi.2014, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Peñasrubias de Pirón (Segovia), 41°8.44'N, 4°7.92'W, 925 m, 21.vi.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Veganzones (Segovia), 41°13.92'N, 4°0.85'W, 890 m, 04.vii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Fuente el Olmo de Fuentidueña (Segovia), 41°24.72'N, 4°1.02'W, 920 m, 10.vii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Ojos Albos (Ávila), 40°41.18'N, 4°28.76'W, 1280 m, 11.vii.2014, J. C. V. and B. P. leg.; Larva on Rosa sp., San Rafael, Sierra de Guadarrama (Segovia), 40°41.35'N, 4°14.56'W, 1260 m, 11.vii.2014, J. C. V. and B. P. leg.; Egg on *Rosa* sp., Aldeanueva de la Serrezuela (Segovia), 41°30.24'N, 3°46.73'W, 1300 m, 15.vii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Navares de las Cuevas (Segovia), 41°24.83'N, 3°46.67'W, 1130 m, 15.vii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Villacastín (Segovia), 40°46.68'N, 4°21.77'W, 1180 m, 09.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Campo Azálvaro (Segovia), 40°41.27'N, 4°21.66'W, 1290 m, 09.viii.2014, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Matabuena, Sierra de Guadarrama (Segovia), 41°3.22'N, 3°46.41'W, 1955 m, 23.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Frumales (Segovia), 41°24.65'N, 4°8.20'W, 865 m, 23.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Dehesa Mayor (Segovia), 41°24.58'N, 4°15.38'W, 860 m, 23.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Cuevas de Provanco (Segovia), 41°35.59'N, 3°53.99'W, 915 m, 30.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Bahabón (Valladolid), 41°29.98'N, 4°15.48'W, 875 m, 30.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Canalejas de Peñafiel (Valladolid), 41°30.06'N, 4°8.29'W, 835 m, 30.viii.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., La Mata (Segovia), 41°8.62'N, 3°46.47'W, 1180 m, 01.xi.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Cantalojas, Sierra de Ayllón (Guadalajara), 41°14.16'N, 3°17.90'W, 1320 m, 06.ix.2014, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Francos, Sierra de Ayllón (Segovia), 41°24.97'N, 3°17.95'W, 1035 m, 06.ix.2014, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Galve de Sorbe (Guadalajara), 41°14.18'N, 3°10.74'W, 1315 m, 06.ix.2014, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Torrecilla del Pinar (Segovia), 41°19.32'N, 4°0.94'W, 875 m, 14.vi.2015, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Castroserna de Abajo (Segovia), 41°14.03'N, 3°46.54'W, 990 m, 06.vii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Castrojimeno (Segovia), 41°30.19'N,

3 53.92'W, 1095 m, 13.vii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Valle de Tabladillo (Segovia), 41°24.78'N, 3°53.84'W, 1025 m, 13.vii.2015, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Pradales (Segovia), 41°30.28'N, 3°39.54'W, 1140 m, 29.vii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Puerto de Malangosto (Madrid), 40°57.76'N, 3 53.48'W, 1930 m, 02.viii.2015, J. C. V. and B. P. leg; Larva on *Rosa* sp., Palazulelos de Eresma, Sierra de Guadarrama (Segovia), 40°52.30'N, 4°0.52'W, 1620 m, 03.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Palazuelos de Eresma, Sierra de Guadarrama (Segovia), 40°57.70'N, 4 0.60'W, 1845 m, 03.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Puerto Alto del León (Madrid), 40°41.43'N, 4°7.46'W, 1525 m, 04.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., El Espinar, Sierra de Guadarrama (Segovia), 40°46.76'N, 4°14.66'W, 1295 m, 04.viii.2015, J. C. V. and B. P. leg; Larva on *Rosa* sp., Revenga, Sierra de Guadarrama (Segovia), 40°52.23'N, 4°7.64'W, 1185 m, 09.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Maderuelo (Segovia), 41°30.32'N, 3°32.35'W, 945 m, 16.viii.2015, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Alconada de Maderuelo (Segovia), 41°24.92'N, 3°32.31'W, 935 m, 16.viii.2015, J. C. V. and B. P. leg.; Eggs and larvae on *Rosa* sp., Aldealengua de Santa María (Segovia), 41°30.35'N, 3°25.16'W, 955 m, 16.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Barahona del Fresno (Segovia), 41°19.51'N, 3°32.26'W, 1010 m, 16.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Cuellar (Segovia), 41°24.50'N, 4 22.55'W, 880 m, 22.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Grado del Pico, Sierra de Ayllón (Segovia), 41°19.58'N, 3°10.75'W, 1355 m, 16.viii.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Ayllón (Segovia), 41°24.95'N, 3°25.13'W, 1015 m, 19.ix.2015, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Soto del Real (Madrid), 40°47.00'N, 3°46.22'W, 1025 m, 06.x.2015, J. C. V. leg.; Larva on *Rosa* sp., Segovia, around the city (Segovia), 40°57.64'N, 4°7.73'W, 955 m, 16.x.2015, J. C. V. and B. P. leg.; Larva on *Rosa corymbifera*, Urueña (Valladolid), 41°45.32'N, 5°13.51'W, 820 m, 07.vii.2016, J. C. V. and B. P. leg.; Larva on *Rosa corymbifera* and *R. micrantha*, La Santa Espina (Valladolid), 41°45.45'N, 5°6.30'W, 815 m, 07.vii.2016, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Montejo de la Vega de la Serrezuela (Segovia), 41°35.69'N, 3°39.60'W, 925 m, 12.vii.2016, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Montejo de la Vega de la Serrezuela (Segovia), 41°35.73'N, 3°32.40'W, 905 m, 12.vii.2016, J. C. V. and B. P. leg.; Larva on *Rosa tomentosa*, San Llorente (Valladolid), 41°40.93'N, 4°1.28'W, 910 m, 28.vii.2016, J. C. V. and B. P. leg.; Larva on *Rosa* sp., Cuevas de Ayllón (Segovia), 41°24.97'N, 3°17.95'W, 1035 m, 20.viii.2016, J. C. V. and B. P. leg. Larvae on *Rosa* sp., Sepúlveda (Segovia), 41°19.43'N, 3°46.60'W, 1025 m, 16.x.2016, J. C. V. and B. P. leg.; Eggs on *Rosa* sp., Renedo de Esgueva (Valladolid), 41°40.52'N, 4°37.31'W, 810 m, 11.vi.2017, J. C. V. leg.; Eggs and larvae (L1) on *Rosa* sp., Montejo de la Sierra (Madrid), 41°8.70'N, 3°32.17'W, 1280 m, 24.vi.2017, J. C. V. and A. García leg.; Eggs and larvae (L1) on *Rosa* sp., Montejo de la Sierra (Madrid), 41°3.30'N, 3°32.13'W, 1340 m, 24.vi.2017, J. C. V. and A. G. leg.; Eggs on *Rosa* sp., La Hiruela (Madrid), 41°3.33'N, 3°24.99'W, 1260 m, 24.vi.2017, J. C. V. and A. G. leg.; Eggs and larvae (L1) on *Rosa* sp., Puerto de la Hiruela (Madrid), 41°3.33'N, 3°24.99'W, 1470 m, 24.vi.2017, J. C. V. and A. G. leg.; Eggs on *Rosa* sp., Puebla de la Sierra (Madrid), 40°57.92'N, 3°24.96'W, 1160 m, 24.vi.2017, J. C. V. and A. G. leg.; Eggs on *Rosa* sp., Berzosa del Lozoya (Madrid), 40°57.89'N, 3°32.09'W, 1025 m, 24.vi.2017, J. C. V. and A. G. leg.

Spialia sertorius (Hoffmannsegg, 1804)

Spain: 1 larva, *ex ovum* on *Sanguisorba minor*, Garganta de Vadillo, Losar de la Vera (Cáceres), 40°7.51'N, 5°38.43'W, 480 m, 22.vi.2006, leg. et coll. J. L. H.-R. (JHSEM-C12P11 and JHSEM-C12P12); 1 pupa, *ex ovum* on *Sanguisorba minor*, Garganta de Vadillo, Losar de la Vera (Cáceres), 40°7.51'N, 5°38.43'W, 480 m, 22.vi.2006, leg. et coll. J. L. H.-R. (JHSEM-C09P01); 4 eggs on *Sanguisorba minor*, Vallelado (Segovia), 41°24.50'N, 4°22.55'W, 815 m, 1.vi.2013, leg. J. C. V., coll. J. L. H.-R. (JHSEM-C20P09);

***Microgaster australis* Thomson, 1895 (Hymenoptera, Braconidae, Microgastrinae)**

Spain: 2 males, Puerto de La Ragua, Sierra Nevada, 37°5.48'N, 3°3.38'W, 2090 m, *ex S. rosae* (L5, L4), 19.vii.2012, em. 1.viii.2012, 23.viii.2012, leg. J. L. H.-R., det. M. Shaw, coll. J. L. H.-R. (JH6569) and National Museums of Scotland (1 leg of JH6569: MRS/JFT 0278, MRS/JFT 0247).

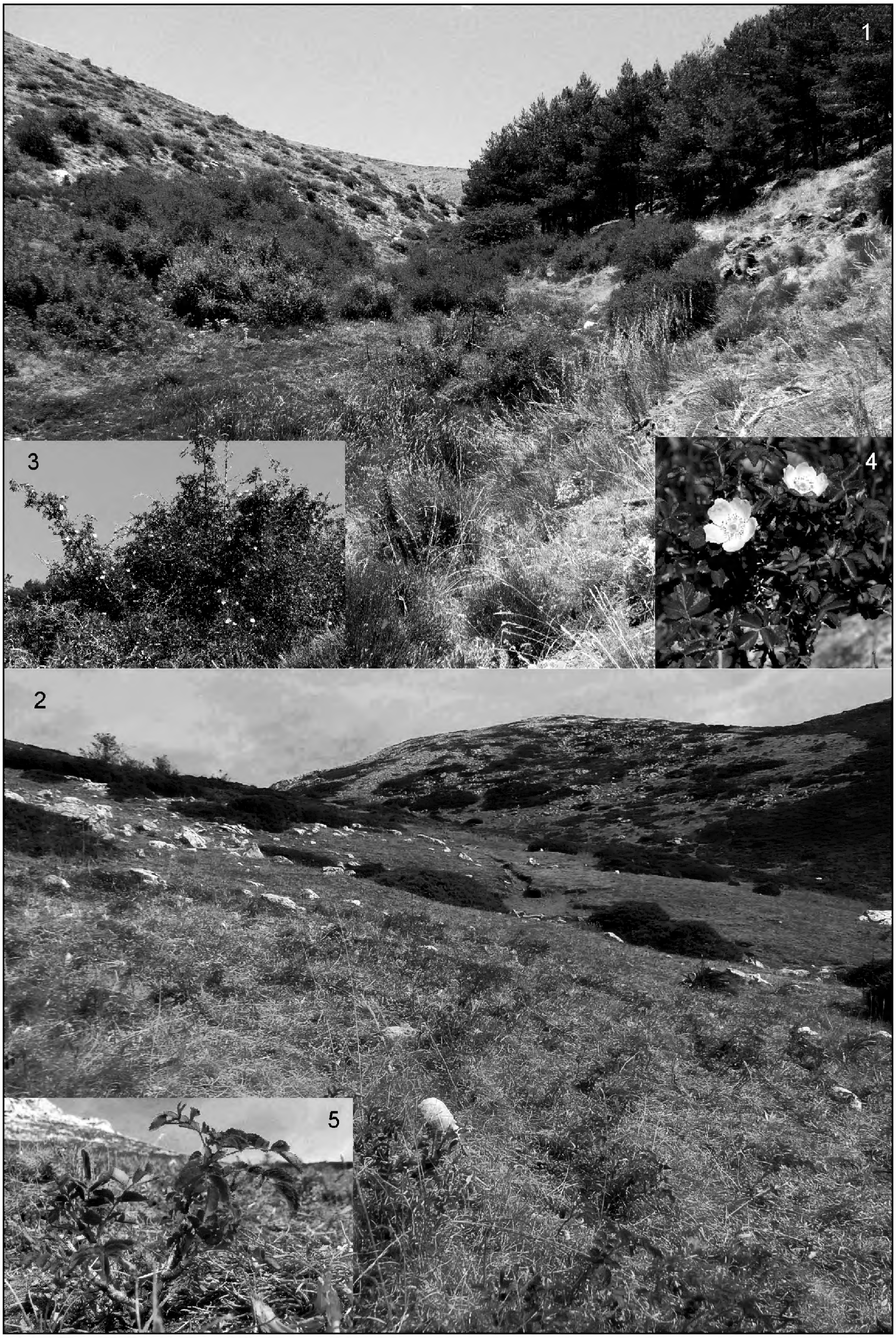
Habitat and natural history in the Iberian Peninsula

Immature stages of *S. sertorius* were found in the wild on *Sanguisorba minor* Scop. (Rosaceae). In the case of *S. rosae*, immature stages were collected on rose bushes. The species of *Rosa* (Rosaceae) on which *S. rosae* has been previously cited in the literature (Hernández-Roldán et al. 2016; Monasterio et al. 2017b) are: *R. elliptica* Tausch, *R. pendulina* L., *R. canina* L., *R. micrantha* Borrer ex Sm., *R. pouzinii* Tratt., *R. sicula* Tratt., *R. agrestis* Savi, *R. agrestis* × *micrantha*, *R. micrantha* × *agrestis* and *R. squarrosa* (Rau) Boreau. We have determined the following rose species used as host-plants of *S. rosae* in several additional localities (see Studied Material): *R. micrantha*, *R. corymbifera* Borkh. and *R. tomentosa* Sm., the last two are new host-plants of *S. rosae*.

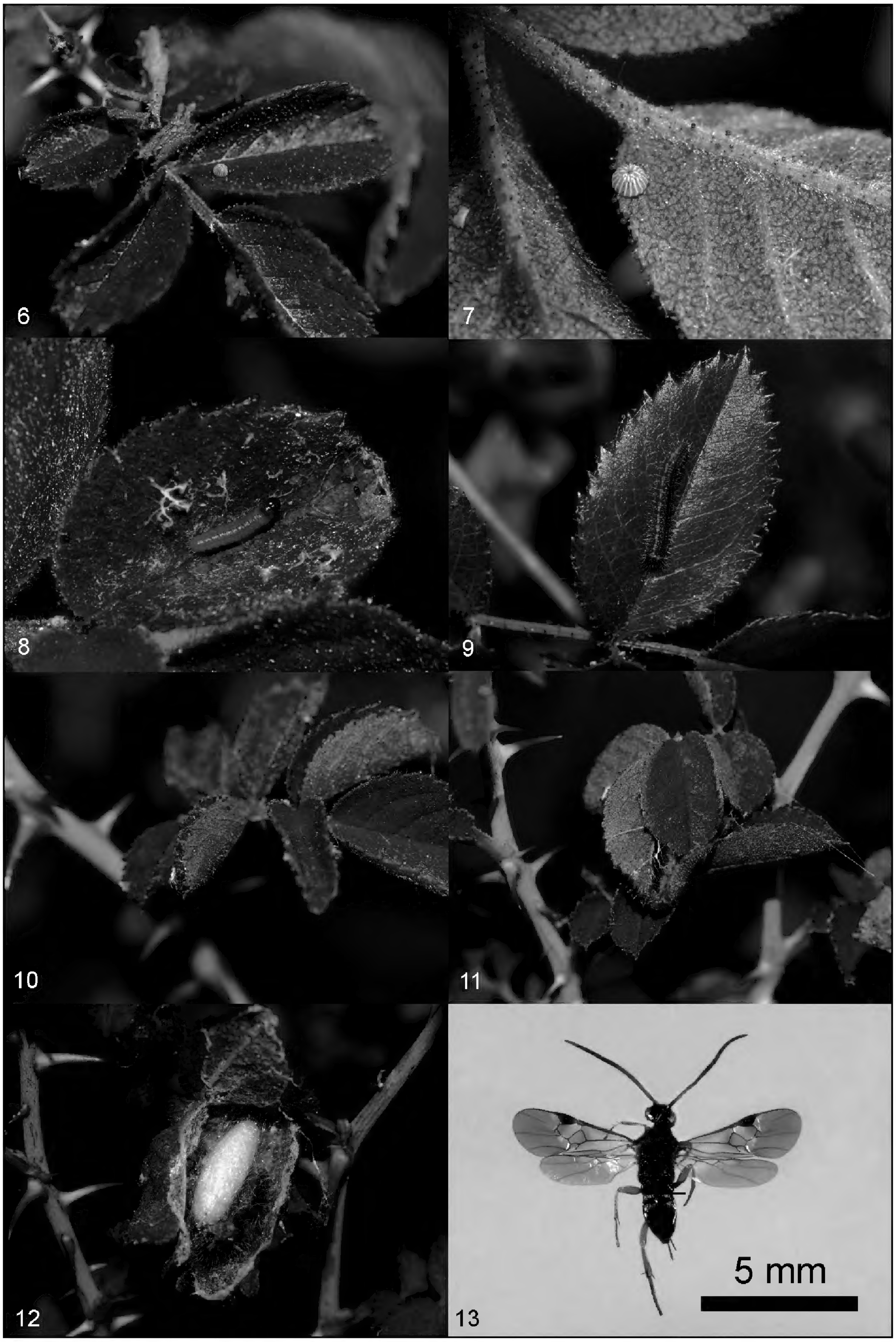
The habitats of *S. rosae* in the Iberian Peninsula are located at altitudes ranging from 469 m (Navarra, N Spain) to 2640 m (Sierra Nevada, SE Spain). The type locality of Puerto de la Ragua (Sierra Nevada, Granada, SE Spain, Fig. 1) is located at high altitude (2000–2100 m) in the oromediterranean bioclimatic belt. This site has *S. rosae*, but not *S. sertorius*. The substrate is formed of Palaeozoic siliceous materials of the Sierra Nevada–Filabres complex and consists mainly of mica schists and quartzites (Molero-Mesa et al. 1992; Sanz de Galdeano 1997). Plant communities belong to the Betic province, Nevada sector (Rivas-Martínez 1987; Rivas-Martínez et al. 2002). The area has a humid to subhumid climate (Molero-Mesa et al. 1992) with 95% of precipitation in the form of snow between November and April and a severe summer drought (Montávez et al. 1996). Average winter and summer temperatures are -2°C and 15°C respectively (González-Megías 2001). Vegetation is dominated by *Erinacea anthyllis* Link (Fabaceae), Fabaceae of the species *Genista versicolor* Boiss. and *Cytisus galianoi* Talavera & P.E. Gibbs, and *Juniperus communis* L. (Cupressaceae) (Molero-Mesa 1988; Molero-Mesa et al. 1992; Valle 2003), and belongs to the *Genisto baeticae–Junipereto nanae* series (Rivas-Martínez 1987). The area has also large expanses of planted pines, mainly of *Pinus sylvestris* L. (Pinaceae).

The habitat of *S. rosae* in the Pico Yordas (Cantabrian Mountains, León, N Spain, Fig. 2) is at an altitude of 1600–1850 m. The substrate consists of Carboniferous limestone rocks. The vegetation belongs to the subalpine belt with lower areas in the montane domain. Plant communities are typical of the Eurosiberian region and the *Daphno cantabricae–Arctostaphyleto* series where *Juniperus communis* and *Arctostaphylos uva-ursi* (L.) Spreng. (Ericaceae) are characteristic species (Rivas-Martínez 1987). Lower parts of the habitat have *Juniperus thurifera* L. as an indicator plant. The shrubs of these communities also include *Rosa pendulina*, one of the larval foodplants of the species. Above 1850 m the vegetation is restricted to grassland with a proportion of bare rock, and the foodplant and the butterfly are absent. *Spialia sertorius* is only found below 1600 m, which is the upper limit of its larval foodplant: *Sanguisorba minor*. At lower altitudes, the vegetation becomes beech forest (*Fagus sylvatica* L., Fagaceae) which is typical of the Eurosiberian montane area.

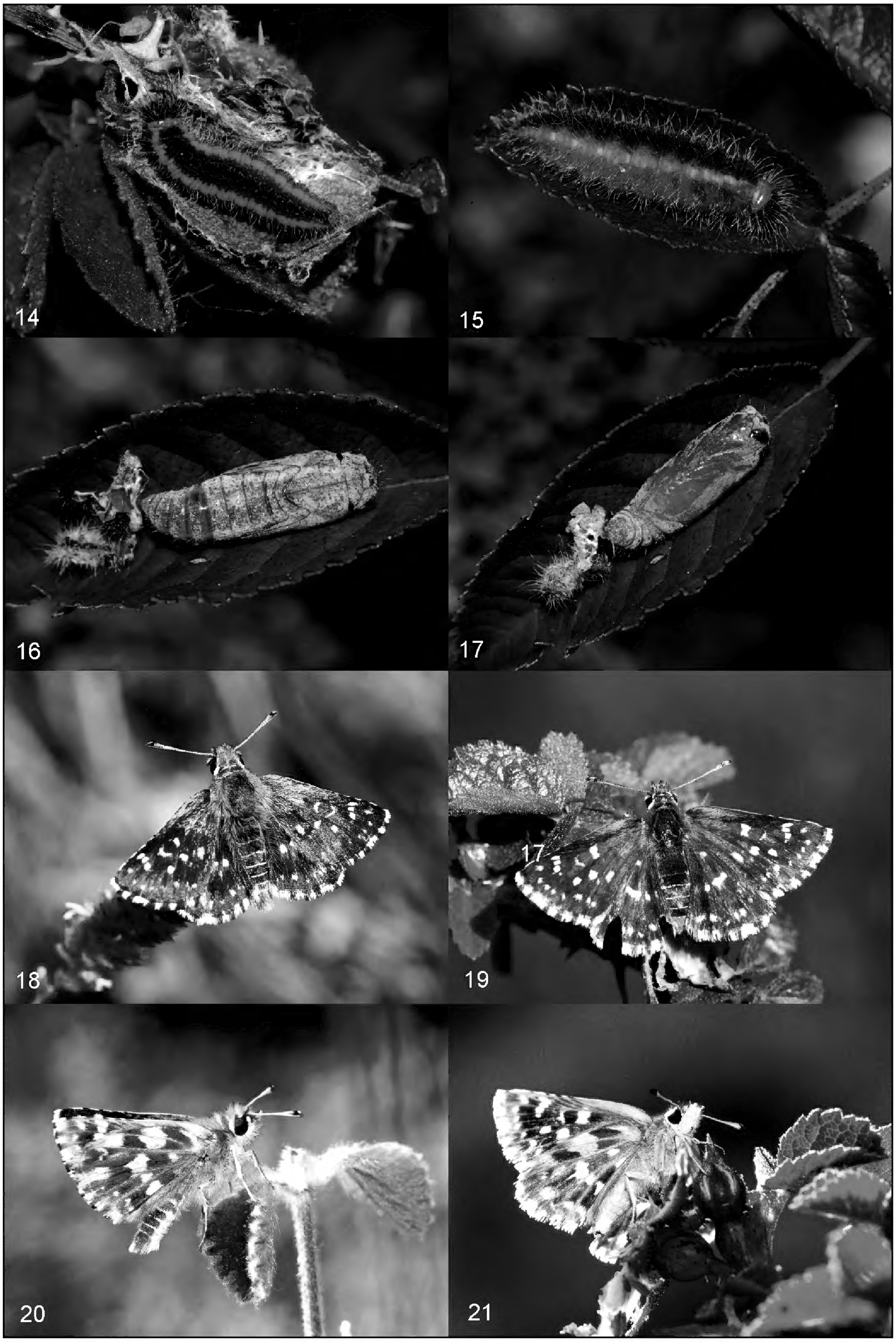
The foodplant of *S. rosae* in Puerto de la Ragua (Granada, SE Spain) is *Rosa sicula* (Figs 3, 4). In this area, at high altitudes, neither *Sanguisorba minor* nor *S. sertorius* were detected. In Pico Yordas (León, N Spain), *S. rosae* feeds on *Rosa pendulina* (Fig. 5), and *S. sertorius* uses *Sanguisorba minor*. Females of *S. rosae* lay their eggs singly on the leaves of roses (Figs 6, 7). After four or five days the larvae have



Figures 1–5. Habitats and foodplants of *Spialia rosae* in the Iberian Peninsula. **1.** Habitat in Puerto de la Ragua, 2090 m (Sierra Nevada, Spain, July 2011). **2.** Habitat in Pico Yordas, 1700 m (Cantabrian Mountains, Spain, September 2012). **3.** Plant of *Rosa sicula* (July 2013). **4.** Flowers of *R. sicula* (July 2013). **5.** Plant of *R. pendulina* (September 2012).



Figures 6–13. Life cycle of *Spialia rosae* on *Rosa sicula* in Puerto de la Ragua, Granada, SE Spain. **6.** Egg on the upperside of a leaf of *R. sicula* and **7.** On the underside of the leaf (July 2012). **8.** Recently emerged first instar larva on a leaf of *R. sicula* (note that the epidermis and parenchyma of the leaf have been eaten, July 2012). **9.** Third instar larva. **10, 11.** Larval shelter made with leaves. **12.** Cocoon of the parasitoid *Microgaster australis* (Hymenoptera), with the remains of a third instar larva of *S. rosae* on *R. sicula*. **13.** Adult of *M. australis* bred from *S. rosae*. Photographs 9–13 were taken in August 2012.



Figures 14–21. Life cycle of *Spialia rosae*. **14.** Dorsal view of a last instar larva on *Rosa sicula* in Puerto de la Ragua, Granada, SE Spain (July 2012). **15.** Pre-pupa on *R. sicula* in Puerto de El Cubillo, Cuenca, Central Spain (August 2012). **16, 17.** Dorsal and lateral views of a pupa on *R. sicula* in Puerto de El Cubillo (September 2012). **18, 19.** Upperside views of adult male and female in Puerto de la Ragua (July 2013). **20, 21**. Side view of adult male and female in Puerto de la Ragua (July 2013).

developed and eclosion takes place (Fig. 8). Larvae feed on the leaves of their foodplants (Fig. 9) and build shelters, by joining leaves of the plant with silk, to protect themselves against predators and parasitoids (Figs 10, 11). The fourth and fifth larval instars are attacked in Puerto de La Ragua by the solitary endoparasitoid *Microgaster australis* Thomson, 1895 (Hymenoptera, Braconidae, Microgastrinae) (Figs 12, 13), whose adult wasp emerges from the larva. *Microgaster* Latreille, 1804 species regularly parasitise skippers of the subfamily *Pyrginae*, as well as the nymphalid *Vanessa atalanta* (Linnaeus, 1758) (Shaw et al. 2009; Hernández-Roldán et al. 2012a). When larvae reach their full development, they pupate inside a shelter made of leaves of the plant or near it (Figs 14, 15). The pupal stage (Figs 16, 17) lasts for 20–22 days (n = 2), after which the adults emerge, in one generation at high altitudes during July–August or in two generations at medium altitudes during May–June and July–August (Figs 18–21).

Immature stage morphology of *Spialia rosae*

Egg (Figs 6, 7, 22–29)

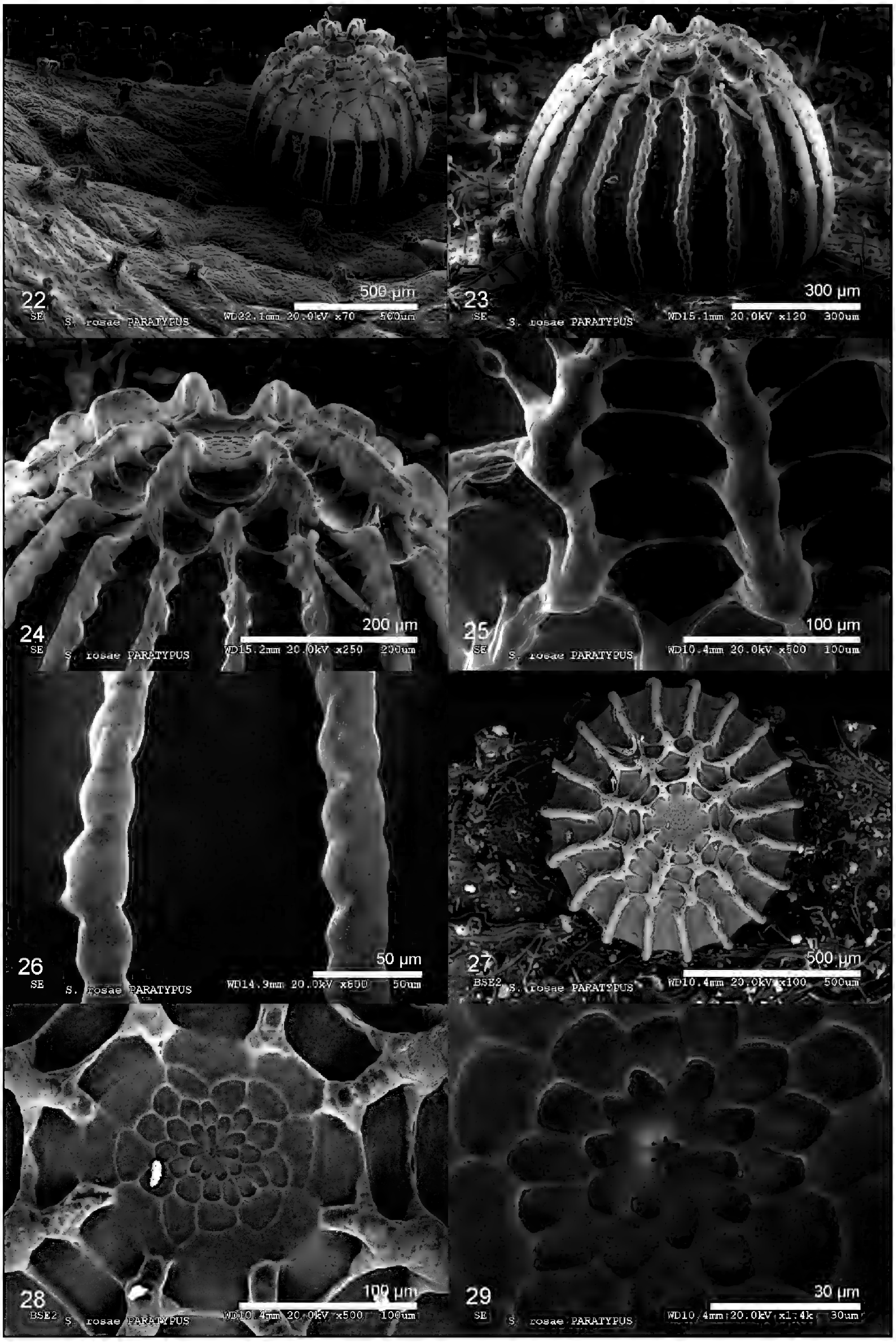
The egg is whitish when laid and turns to pale orange in 24–48 hours. Its form is spherical with depressions in the base and annular areas. The diameter is 0.63–0.69 mm (number of samples examined, n = 2) and its height 0.55–0.57 mm (n = 2). The chorion surface is perforated by pores (Fig. 26) and the egg is covered with radial and transverse ribs that form cells, usually with a rectangular form (Figs 25, 26). Where radial ribs meet in the proximity of the annular area the cells have a pentagonal form (Fig. 23). Aeropyles (respiratory openings of the egg) are found on the crossing of radial and transverse ribs (Figs 25, 26). At the equator of the egg the number of radial ribs is 19–20 (n = 2, Fig. 27). The radial ribs are much higher (29 ± 4 μm, n = 3) than the transverse ribs (Fig. 26). In the annular area of the egg, the micropylar rosette has nine to ten polygonal petaloid cells (Fig. 29). Surrounding the micropylar rosette there are three series of cells, resulting in a total number of cells in this area of 40 to 43 (n = 2, Fig. 28). The micropylar depression is hexagonal with six micropylar openings in the angles (Fig. 29).

Last instar larva (Figs 14, 15, 30–37)

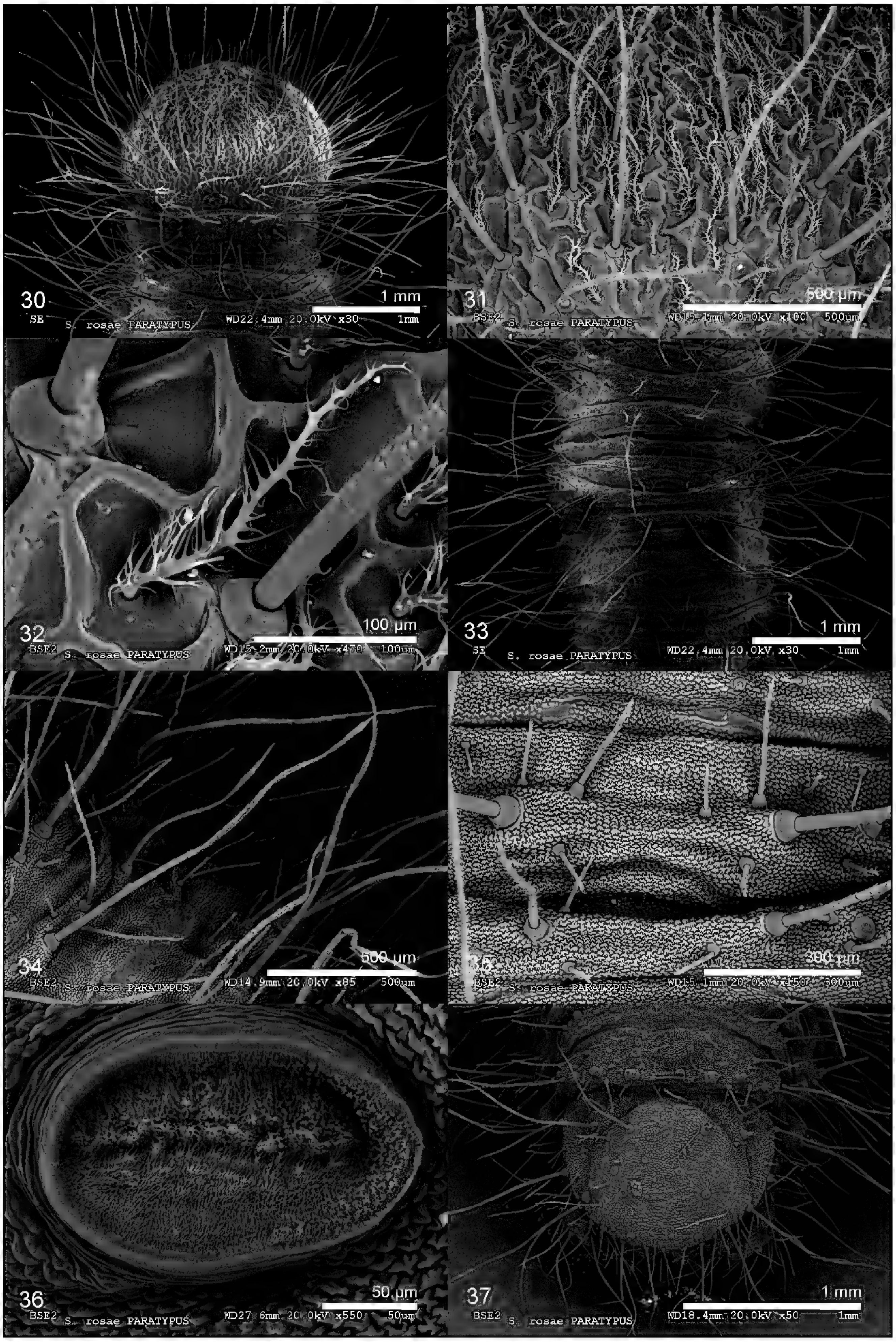
The surface of the head capsule displays a reticular pattern and is covered with a large number of highly branched setae and a few longer and smooth setae with a pointed tip (Figs 31, 32). The branched setae seem to be unique to *Spialia* within the Hesperiidae. The collar is placed after the head capsule and it has a smooth cuticle and setae of different lengths (Fig. 30). In the rest of the body segments the cuticle is armoured by star-like formations (Fig. 35). A pair of trichobothria is found at each side of the lateral zone on the first thoracic segment (Fig. 33). Each segment of the thorax, the abdomen and the collar have a couple of special barrel-like cuticular formations (Fig. 35) described for the first time by Hernández-Roldán et al. (2011) in the genus *Pyrgus*. The dorsal and subdorsal areas of each segment bear narrow setae of different sizes with pointed tips and a rounded base (Figs 34, 35). Spiracles are placed between the subdorsal and lateral areas on the third thoracic and on the abdominal segments (Fig. 36). The inner part of the spiracles has branched papillae. The anal plate (Fig. 37) has a smooth surface and has pointed-tipped setae of different lengths.

Pupa (Figs 16, 17, 38–45)

The pupa is fusiform, with a smooth cuticle that is dark grey or black. It is covered in wax, giving the pupa a characteristic appearance, with a fine powder on its surface and a light grey colour (Figs 16, 17). It has two mesothoracic tubercles on both sides of the anterior border of the mesothorax



Figures 22–29. Scanning electron microscope images of the egg of *Spialia rosae* (Paratypes from Puerto de la Ragua, Granada, SE Spain). **22.** Lateral view of egg on a leaf of *Rosa sicula* showing the glands of the plant epidermis. **23.** Lateral view. **24.** Lateral view of the annular area with annular area in the middle. **25.** Cells from the lateral zone of the egg formed by radial and transverse ribs. **26.** Detail of the radial ribs. **27.** View of the annular pole. **28.** Annular area with the micropylar rosette. **29**. Detail of the micropylar rosette and the micropyle showing six micropylar openings.



Figures 30–37. Scanning electron microscope images of a last instar larva of *Spialia rosae* (Paratype from Sierra de Albarracín, Teruel, Central Spain). **30.** Head capsule and collar. **31, 32.** Detail of the head capsule setae and sculpture. **33.** Cuticular formations on thoracic and abdominal segments. **34.** Detail with different types of subdorsal and lateral setae on the first thoracic segment: long and short setae with pointed tips. **35.** Cuticular formations on an abdominal segment. **36.** Last abdominal spiracle. **37.** Last abdominal segments with the anal plate and setae with pointed tips and rounded bases.

(Figs 38, 39). The lateral anterior wall of the tubercles is undulated (Fig. 38). The surface of the rest of the tubercle is setose (Figs 38, 39). The thoracic prespiracular chamber is setose (Fig. 39). The pupal cuticle has scattered setae of different lengths that have a smooth surface, pointed tip and a rounded base. The density of setae is higher on the dorsal areas and in the lateral abdominal zones (Figs 40, 43). The subdorsal zone of the abdominal segments has spiracles (Fig. 41) that are interiorly filled with branched spiny papillae (Fig. 42). The last abdominal segment has a cremaster, formed by hooked setae whose tips are helicoidal (Figs 43, 44). The hooks are used by the pupa for attaching to the silken threads spun by the larva to the substrate (Fig. 45).

Immature stage morphology of *Spialia sertorius*

Immature stages of *S. sertorius* do not have significant diagnostic differences from those of *S. rosae* in the Iberian populations studied.

Egg (Figs 46–53)

This is similar to the egg of *S. rosae*. The diameter is 0.61 ± 0.02 mm (n = 4) and its height 0.51 ± 0.01 mm (n = 3). In the equator of the egg the number of radial ribs is 17–21 (n = 4, Fig. 51). The radial ribs are much higher (23 ± 5 µm, n = 3) than the transverse ribs (Figs. 50, 51). In the annular area of the egg, the micropylar rosette has ten to eleven (n = 2) polygonal petaloid cells (Fig. 53). Surrounding the micropylar rosette there are three series of cells, so that the total number of cells in this area is 37 to 43 (n = 2, Fig. 52), overlapping the range of this feature in *S. rosae*. The micropylar depression is hexagonal with five to six (n = 4) micropylar openings in the angles (Fig. 53).

Last instar larva (Figs 54–61)

The larva of *S. sertorius* (Figs 54–61) is similar to that of *S. rosae* (Figs 30–37).

Pupa (Figs 62–69)

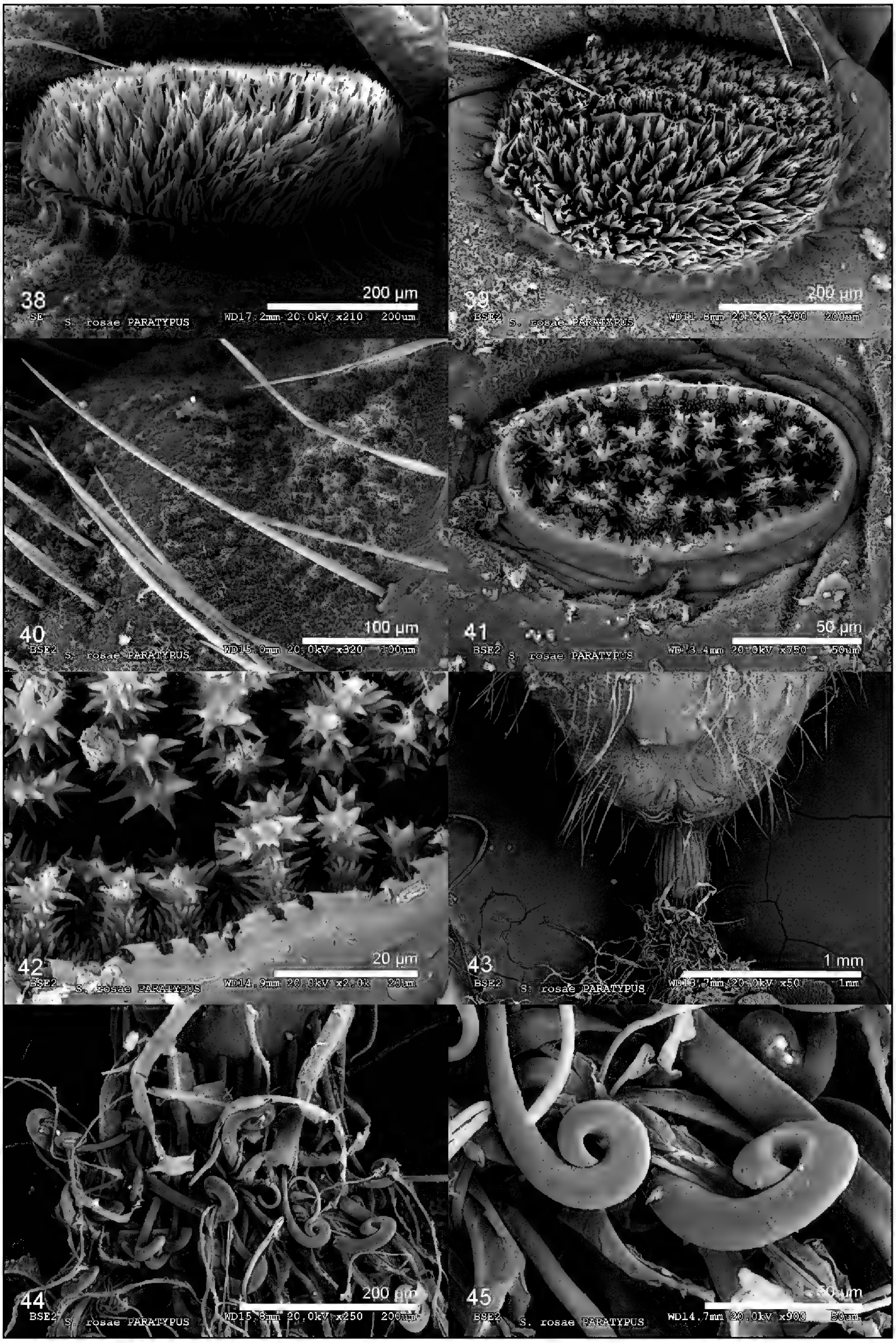
The morphology of the pupa of *S. sertorius* is similar to that of *S. rosae* (Figs 38–45).

Adult external morphology

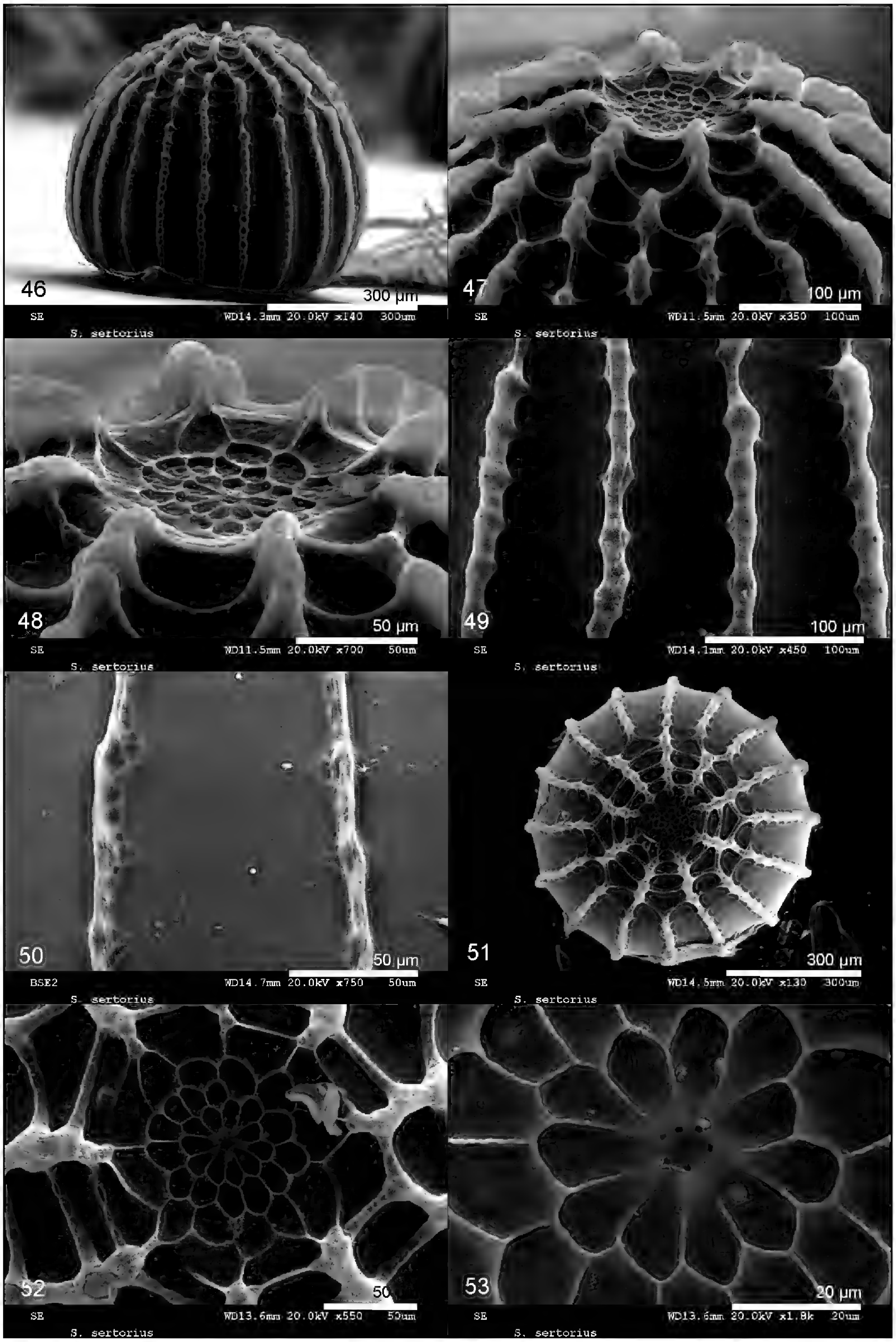
S. rosae and *S. sertorius* are morphologically identical externally as adults. The upperside of the wings in both males and females is dark brown, almost black, with chequered fringes. The upperside of the forewing has a white quadrilateral discal spot, a series of white postdiscal spots (spots in E4 and E5 are often missing or very small), and a submarginal series of small dots. The upperside of the hindwing has a white spot in the cell, an incomplete postdiscal series with only two or three white dots distally, and a series of small submarginal dots. The underside of the forewing has pale or reddish costal, apical and marginal areas, while the discal and postdiscal areas are dark grey or brown, with the same dorsal white spots as on the upperside. The colour of the underside of the hindwing is brick red or reddish ochre, with a series of white postdiscal spots that are larger in E7 and E8. The white spot in E8 is displaced towards the base of the wing, giving the series a curved shape. The submarginal series has larger spots in E4, E5 and E7 (according to the original description in Hernández-Roldán et al. 2016).

Geographic distribution of *Spialia rosae*

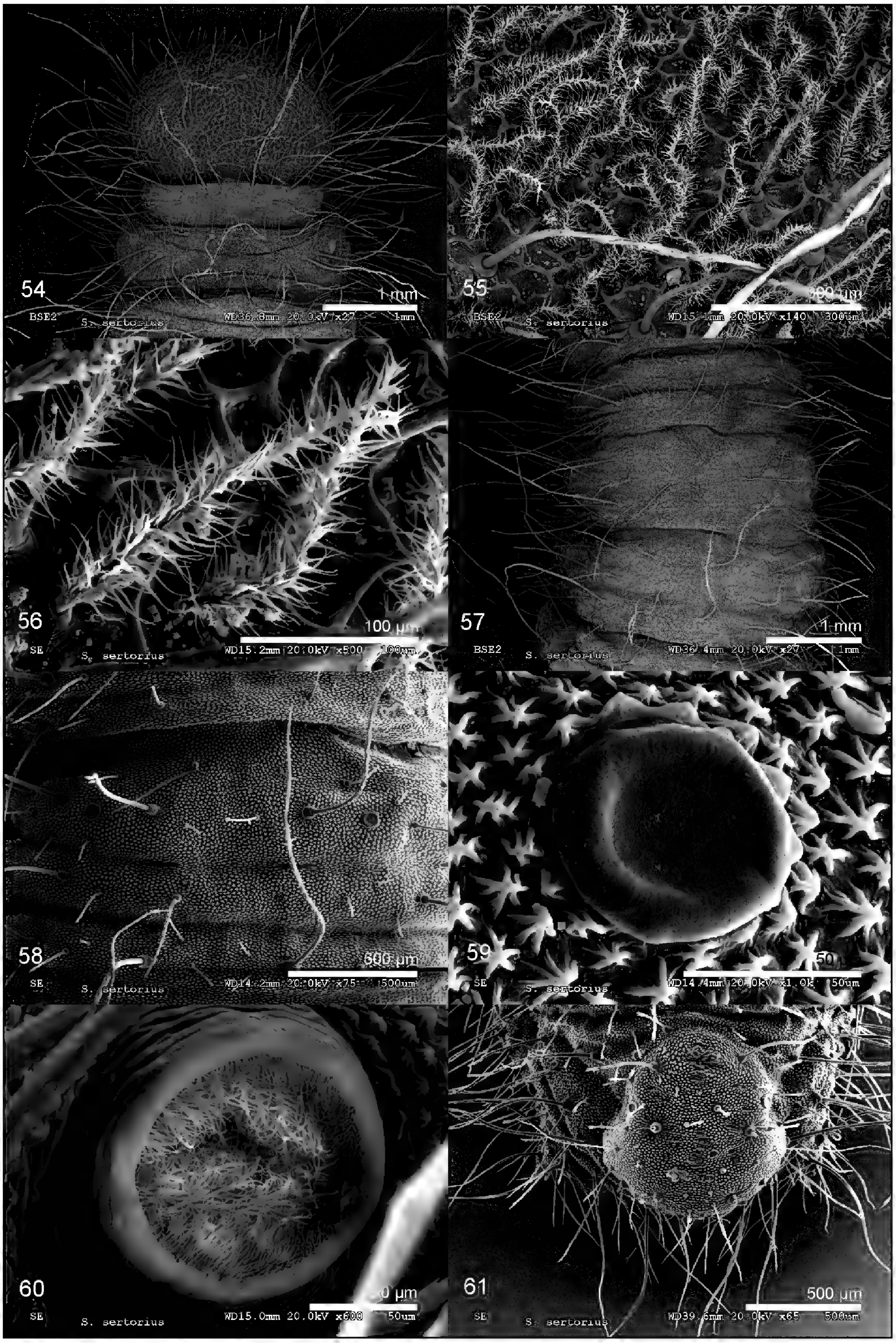
Fig. 70 shows the known distribution of *S. rosae*, which is restricted to the Cantabrian Mountains, the Pyrenees and the Iberian, Central and Betic Mountain Systems. However, our new records,



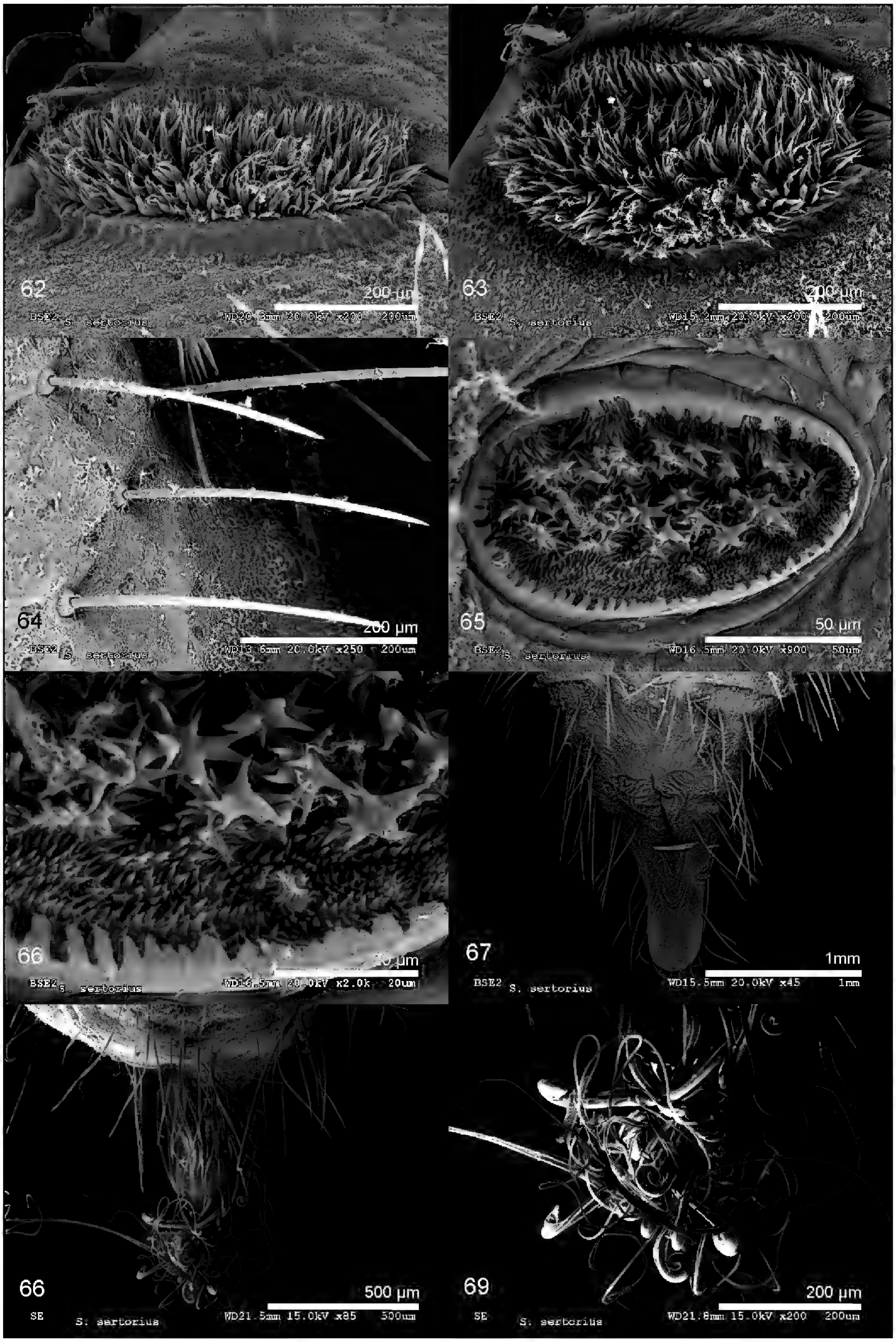
Figures 38–45. Scanning electron microscope images of a pupa of *Spialia rosae* (Paratype from Puerto de El Cubillo, Cuenca, Central Spain). **38.** Lateral view of the mesothoracic tubercle. **39.** Upper view of the mesothoracic tubercle. **40.** Detail of the pupal cuticle showing smooth setae with rounded bases. **41.** Abdominal spiracle. **42.** Detail of the spiny papillae inside the spiracle. **43.** Ventral view of the last abdominal segments. **44.** Detail of the cremaster. **45.** Tips of the cremastral hooks.



Figures 46–53. Scanning electron microscope images of an egg of *Spialia sertorius* from Vallelado, Segovia, Central Spain. **46.** Lateral view. **47.** Lateral view of the annular area. **48.** Detail of the annular area. **49.** Cells of the egg equator formed by radial and transverse ribs. **50.** Detail of the radial ribs. **51.** View of the annular pole. **52.** Annular area with the micropylar rosette. **53.** Micropylar rosette and micropyle showing six micropylar openings.



Figures 54–61. Scanning electron microscope images of a last instar larva of *Spialia sertorius* from Losar de la Vera, Cáceres, Central Spain. **54**. Head capsule and collar. **55, 56.** Detail of the head capsule setae and sculpture. **57.** Cuticular formations on thoracic and abdominal segments. **58.** Detail with different types of subdorsal and lateral setae on the first thoracic segment: long and short setae with pointed tips. **59.** Detail of a barrel-like formation and the cuticular sculpture. **60** Last abdominal spiracle. **61.** Last abdominal segments with the anal plate and setae with pointed tips and rounded bases.



Figures 62–69. Scanning electron microscope images of a pupa of *Spialia sertorius* from Losar de la Vera, Cáceres, Central Spain. **62.** Lateral view of the mesothoracic tubercle. **63.** Upper view of the mesothoracic tubercle. **64.** Detail of the pupal cuticle showing smooth setae with rounded bases. **65.** Abdominal spiracle. **66.** Detail of the spiny papillae inside the spiracle. **67.** Ventral view of the last abdominal segments. **68.** Detail of the cremaster **69.** Detail of the cremastral hooks.

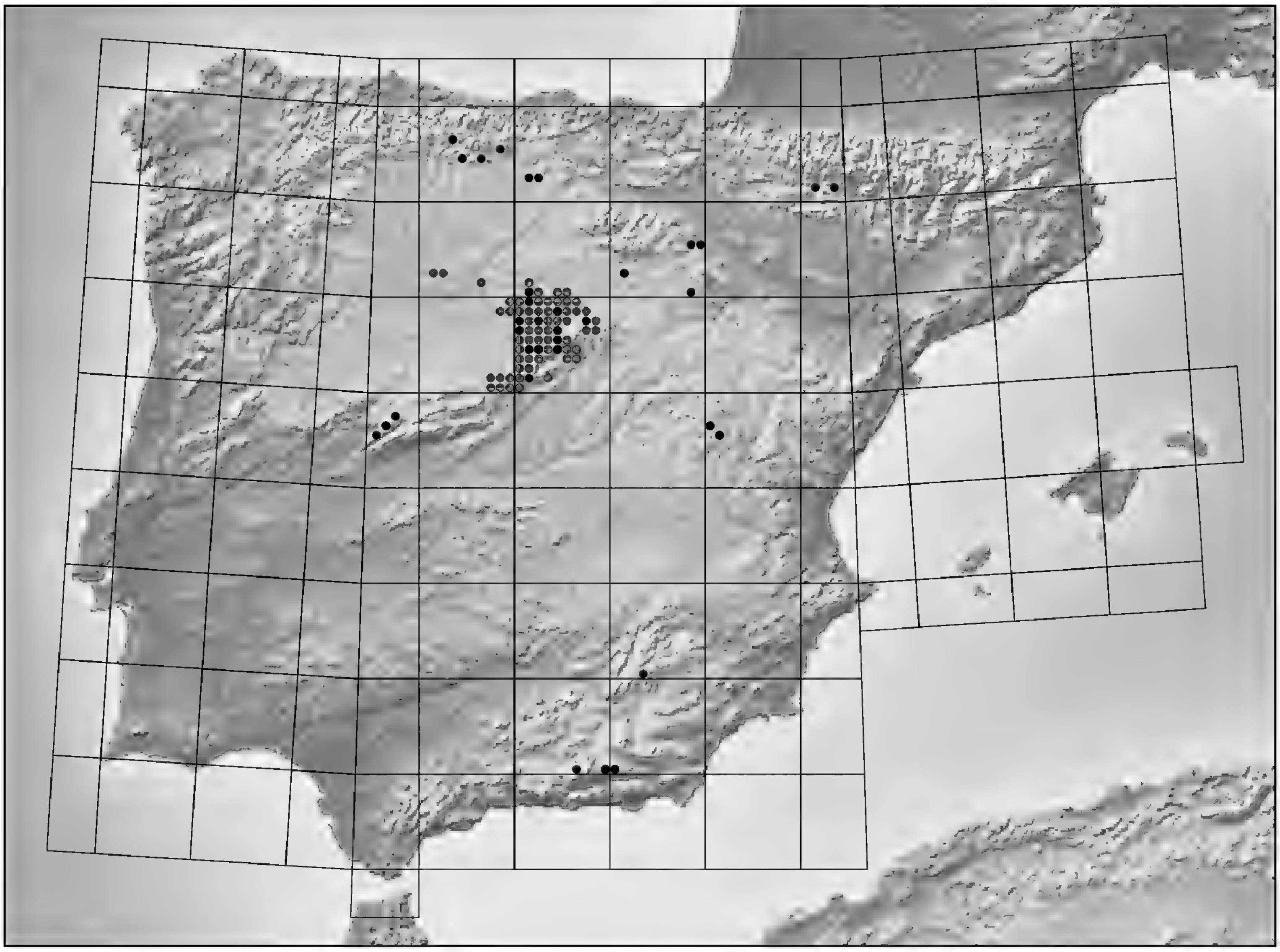


Figure 70. Distribution map of *Spialia rosae* in the Iberian Peninsula using 10 × 10 km squares of the MGRS system. Black circles represent records from the literature and green circles represent new records. Grid represents UTM. 100 × 100 km, symbol size is 10 km.

based mainly on the thorough study in the province of Segovia, show that the species is potentially widespread in the northern part of the interior plateau in Central Spain. Published records gave the presence of the species in 36 MGRS squares (10 × 10 km) while the current map provides evidence for 56 new locations (see studied material) with a total of 92 occupied squares, which represents a 155% increase from previous knowledge. A complete list of the new locations, both with MGRS and geographic coordinates is given in Supplementary Material.

Discussion

Habitat and natural history of *Spialia rosae* and *S. sertorius* in the Iberian Peninsula

The distributions of both species of *Spialia* are clearly related to the altitudinal zonation where their host-plants occur. In Sierra Nevada, as an example of the Mediterranean biogeographic region, *S. sertorius* is present in the thermomediterranean (0–600 m), mesomediterranean (600–1500 m) and supramediterranean (1500–1900 m) belts, and is generally absent from the oromediterranean (1900–2900 m) and crio-oromediterranean (>2900 m) belts (terms for mediterranean biogeographic belts follow Rivas-Martínez 1987). On the other hand, *S. rosae* is present in the supramediterranean and oromediterranean belts, and apparenly absent in the lower ones. Thus, both species can coexist in the supramediterranean belt in this mountain range.

In the Cantabrian Mountains, which belong to the Atlantic biogeographical region, *S. sertorius* is present in the lowest bioclimatic belt (*Quercus robur* L. (Fagaceae) and *Q. ilex* L. forests, 0–500 m) and montane belt (*Fagus sylvatica* (Fagaceae) and other deciduous forests, 500–1700 m), while *S. rosae* is present in the montane and subalpine belts (heathlands, 1700–2200 m) and probably also in the alpine belt (grasslands above 2200 m). Here, the two species can coexist in the montane belt.

Differences in the immature stage morphology of *Spialia* and *Pyrgus* species

We compared the morphological characters of the immature stages of *S. rosae* and *S. sertorius* (which do not present constant diagnostic differences between the two species) with those of the European species of the genus *Pyrgus*, which have been extensively studied (Hernández-Roldán et al. 2011, 2012a, 2012b), belong to the same subfamily *Pyrginae* and are phylogenetically close to the genus *Spialia*. The eggs of *Spialia* are smaller (diameter = 0.61–0.66 mm) than those of *Pyrgus* (lower tertile diameter < 0.72 mm, minimum values in *Pyrgus onopordi* (Rambur, 1839) and *P. serratulae* (Rambur, 1839): 0.66 mm). When considering their height, eggs of *Spialia* (height = 0.51–0.56 mm) are lower than those of *Pyrgus* (lower tertile, height < 0.57 mm), and the radial ribs can be classified as tall (23–29 µm; higher tertile in *Pyrgus* > 12 µm). At the equator of the egg the number of radial ribs (17–21) is low (lower tertile in *Pyrgus* < 21). The total number of cells surrounding the micropylar rosette (37 to 43) is close to the medium range of *Pyrgus*, where the central tertile is 39–46.

Larvae of *Spialia* in the examined Iberian samples, have highly branched setae on the head capsule, while this type is not found on the head capsule of the last instar larvae of *Pyrgus*. On the other hand, narrow setae with their tip forming a crown, which are present in *Pyrgus,* are missing on the last instar larvae of *Spialia*. In addition, both genera have two characteristic cuticular barrel-like formations on each segment of the thorax, abdomen and on the collar. The inner part of the spiracles in *Spialia* has branched papillae, while most of the species of *Pyrgus,* with the exception of *P. andromedae* (Wallengren, 1853), *P. cacaliae* (Rambur, 1839), *P. malvae* (Linnaeus, 1758) and *P. malvoides* (Elwes & Edwards, 1897), have filiform spiracular papillae.

The pupae of *Spialia* have the mesothoracic tubercles with an undulated external wall and a hairy surface, features that are found in most species of *Pyrgus*, except for the external wall of the mesothoracic tubercles, which is smooth in *P. malvae* and *P. malvoides*, and the surface of the rest of the tubercle, which is smooth in *P. malvoides*, covered with spines in *P. malvae*, and rugose in *P. andromedae* and *P. cacaliae*. The pupal spiracles of *Spialia* have branched papillae in their interior as do most species of *Pyrgus*, except in *P. andromedae*, *P. cacaliae*, *P. malvae* and *P. malvoides,* which have unbranched spiracle papillae.

Geographic distribution of *Spialia* species in the Iberian Peninsula

While *S. sertorius* is widely distributed in the Iberian Peninsula except at the highest altitudes, *S. rosae* is restricted to mid and high elevations. Thus, their distributions are complementary, with a wide overlap at mid-mountain. Nevertheless, it is important to note that the status of the two species is radically different because:

1) *S. sertorius* is widely distributed in Western Europe and *S. rosae* is apparently endemic to the Iberian Peninsula.

2) While *S. sertorius* is present in all the regions of the Iberian Peninsula, *S. rosae* seems to be absent in some mountains, for example the Eastern Pyrenees, where it has been searched for without success.

3) The populations of *S. sertorius* are generally connected through lowland habitat, as this species tolerates anthropogenic effects quite well.

In the case of *S. rosae*, most populations in mountain systems are fragmented. Nevertheless, the discovery of the existence of this species in the northern part of the interior plateau, and potentially quite widespread, suggests that the populations of the Cantabrian Mountains could be connected to those of the Central Mountain System and Northern Iberian Mountain System. Moreover, the fact that the species has been found in most places in which it was searched for in the Province of Segovia predicts a wider distribution than that shown by our map. It would be very important to assess if *S. rosae* is indeed widespread in the whole northern part of the interior plateau, as well as testing its potential presence in the southern part of the interior plateau and other parts of the Iberian Peninsula where it has not yet been recorded.

Conservation

Spialia rosae has an apparently relatively limited and fragmented distribution in the Iberian Peninsula and prefers mountain areas (Fig. 70). Its preference for montane habitats could be a threat to the populations of the species due to the possible effects of climate change (Wilson et al. 2007). Based on the current data, we are certain that this species does not meet any of the IUCN threat categories based on geographical criteria, neither regarding the extent of occurrence, which is clearly more than 20000 km^2, nor with respect to the area of occupancy, which is more than 2000 km^2 (criteria B, IUCN 2001). Our study has shown that an increase of sampling effort in favourable areas can substantially increase the known distribution of this butterfly. At the moment it is not possible to rely on population abundance or trends to analyse the species' conservation status (criteria A, C, D or E, IUCN 2001). In the future it will be important to monitor whether the populations of the species show positive, negative or stable trends. This would only be possible if the monitoring programme that is already taking place in many parts of Spain (Spanish Butterfly Monitoring Scheme) is capable of providing data for the species. Transects already taking place in the Cantabrian Mountains, Central Mountain System and Sierra Nevada will indeed be important in producing trend data. In conclusion, we change the previous category of Data Deficient (DD) (Hernández-Roldán et al. 2016) to Least Concern (LC), based on the new data provided by this paper, and encourage studies on the biology, distribution and population trends to fill the gaps in our knowledge on this recently described butterfly.

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Supplementary material 1

List of the new locations

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